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Biological grading of breast cancer using antibodies to proliferating cells and other markers.

Bacus SS, Goldschmidt R, Chin D, Moran G, Weinberg D, Bacus JW.

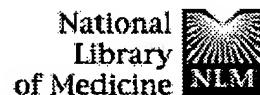
Cell Analysis Systems, Inc., Lombard, IL 60148.

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Quantitation of immunohistochemical staining by image analysis was performed on 50 breast cancers stained with the monoclonal antibody Ki-67 to determine the growth fraction and its correlation with tumor grade. A high degree of correlation was shown. For each case the DNA ploidy was determined by quantitation of the DNA Feulgen stain by computerized microdensitometry. DNA content of breast tumor cells correlated to the histopathologic grade at which poorly differentiated tumors are more likely to be aneuploid. Quantitation of immunohistochemistry for estrogen and progesterone receptors had a high degree of correlation with the steroid binding assay, such as dextran-coated charcoal assay (DCCA), and were weakly correlated to histologic grade. In summary, our results indicated that quantitation of Ki-67-positive nuclear area and of DNA content by image analysis provides an objective method for assessing tumor cell growth fraction and DNA ploidy. Quantitation of steroid receptors by immunohistochemistry is a better and easier technique than those currently used to determine the best therapy for postmenopausal women. These methods can be performed on small frozen sections or needle aspirates in quantities that are insufficient for current steroid binding assays. Thus, this method is prognostically useful even for patients with small breast lesions.

PMID: 2817079 [PubMed - indexed for MEDLINE]

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Neu differentiation factor (heregulin) induces expression of intercellular adhesion molecule 1: implications for mammary tumors.

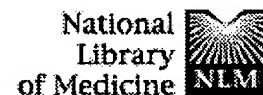
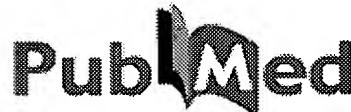
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Bacus SS, Gudkov AV, Zelnick CR, Chin D, Stern R, Stancovski I, Peles E, Ben-Baruch N, Farbstein H, Lupu R, et al.

Cell Analysis Systems, Inc., Elmhurst, Illinois 60126.

Related Resources

Neu differentiation factor (NDF, also called heregulin) is a 44-kilodalton glycoprotein that stimulates tyrosine phosphorylation of the Neu/HER-2 receptor and induces phenotypic differentiation of certain mammary cancer cell lines to growth-arrested and milk-producing cells. To determine which molecules participate in the concomitant morphological alterations, we analyzed the expression of several cytoskeletal and surface molecules and found that NDF elevated the expression of the intercellular adhesion molecule 1 (ICAM-1) in cultured AU-565 human adenocarcinoma cells. The levels of both the protein and the mRNA of ICAM-1 were elevated after 3-5 days of treatment with NDF. Elevated expression of ICAM-1 was induced also by gamma-interferon and by the tumor-promoting phorbol ester (PMA), albeit with different kinetics. Down-regulation of protein kinase C or its inhibition by calphostin C partially inhibited the effect of NDF, implying that the induction of ICAM-1 may be mediated by protein kinase C. NDF transcripts were detectable in 3 of 9 human mammary tumors, suggesting that the *in vitro* effect of the factor may be relevant to breast cancer. By selecting Neu-positive human mammary tumors ($n = 39$), we found a significant correlation ($P < 0.001$) between the expression of ICAM-1 and histological features of invasive ductal carcinoma with a prominent carcinoma *in situ* component. When cultured *in vitro* the cells of these tumors grew in clusters and formed domelike structures reminiscent of comedo-type carcinoma *in situ*. In addition, the majority of patients with tumors that coexpressed ICAM-1 and Neu had no lymph node involvement, unlike most Neu-positive but ICAM-1-negative tumors, which metastasized to the lymphatic system. Taken together, our observations suggest that the induction of ICAM-1 by NDF may affect the morphology, differentiation state, and metastasis of Neu-expressing mammary tumor cells.



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1: Am J Clin Pathol. 1994 Oct;102(4 Suppl 1):S13-24. [Related Articles](#), [Links](#)

Expression of the erbB-2 family of growth factor receptors and their ligands in breast cancers. Implication for tumor biology and clinical behavior.

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Bacus SS, Zelnick CR, Plowman G, Yarden Y.

Advanced Cellular Diagnostics, Inc., Elmhurst, Illinois.

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Transmembrane receptor tyrosine kinases that bind to growth factors transmit signals that are essential to growth and differentiation. These receptors can be classified into groups based on their structure. One group implicated in the pathogenesis of breast cancer contains receptors belonging to the erbB family. This group includes the epidermal growth factor receptors, the HER-2/neu (erbB-2), HER-3, and HER-4. Despite the structural similarity of these receptors, HER-2/neu, HER-4, and HER-3 do not bind to any ligand of the epidermal growth factor receptor. However, a 44-kD glycoprotein called neu differentiation factor (neu differentiation factor/hereregulin) has been isolated. This ligand phosphorylates the HER-2/neu receptor and binds directly to HER-4 and HER-3. The abundance of erbB receptors and their ligands in breast cancers points to their functional importance in the pathogenesis and biological behavior of breast cancers. Furthermore, these receptors and ligands may hold a promise for targeted therapy for breast cancer in the future.

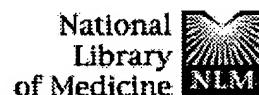
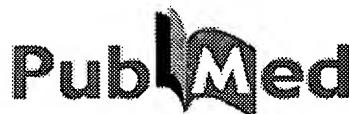
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1: *Cancer Res.* 1997 Dec 1;57(23):5217-20.

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Intracellular localization of p53 tumor suppressor protein in gamma-irradiated cells is cell cycle regulated and determined by the nucleus.

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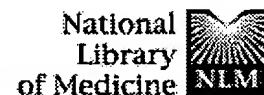
Komarova EA, Zelnick CR, Chin D, Zeremski M, Gleiberman AS, Bacus SS, Gudkov AV.

Department of Molecular Genetics, University of Illinois, Chicago 60607, USA.

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DNA damage leads to the stabilization of p53 protein and its translocation to the nucleus, resulting in activation or suppression of p53-responsive genes. However, a significant proportion of cell nuclei remain negative for p53 and p53-inducible cyclin-dependent kinase inhibitor p21waf1 after a single dose of gamma-irradiation. Quantitation of DNA content in p53-positive and -negative nuclei 4-6 h after 10 Gy of gamma-irradiation of human breast carcinoma MCF7 cells, fibrosarcoma HT1080 cells, and diploid skin fibroblasts showed that p53 and p21waf1 nuclear accumulation occurs predominantly in the G1 phase and at the beginning of the S phase of the cell cycle. The majority of the nuclei in late S phase and in G2-M phase remained p53- and p21waf1-negative. This suggests that there is a cell cycle window during which p53 can accumulate in the nucleus and activate expression of p21waf1. To determine whether cell cycle-dependent distribution of p53 is caused by cytoplasmic modifications of p53 protein or by properties of the nucleus, p53 localization was analyzed in multinucleated cells obtained by polyethylene glycol-mediated cell fusion. Dramatic differences in p53 accumulation were found among the nuclei in individual multinucleated cells. Distribution of p53-positive and -negative nuclei among the phases of the cell cycle was similar to that observed in a regular cell population. These results suggest that the observed differences in p53 accumulation in the nuclei of irradiated cells are determined by cell cycle-dependent nuclear functions. In contrast to p53, p21waf1 was equally distributed among the nuclei of multinucleated cells regardless of the stage of the cell cycle, indicating that the observed phenomenon is specific for p53.

PMID: 9393737 [PubMed - indexed for MEDLINE]



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1: Mol Pharmacol. 2002 Mar;61(3):524-32.

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Endothelin-1 protects ovarian carcinoma cells against paclitaxel-induced apoptosis: requirement for Akt activation.

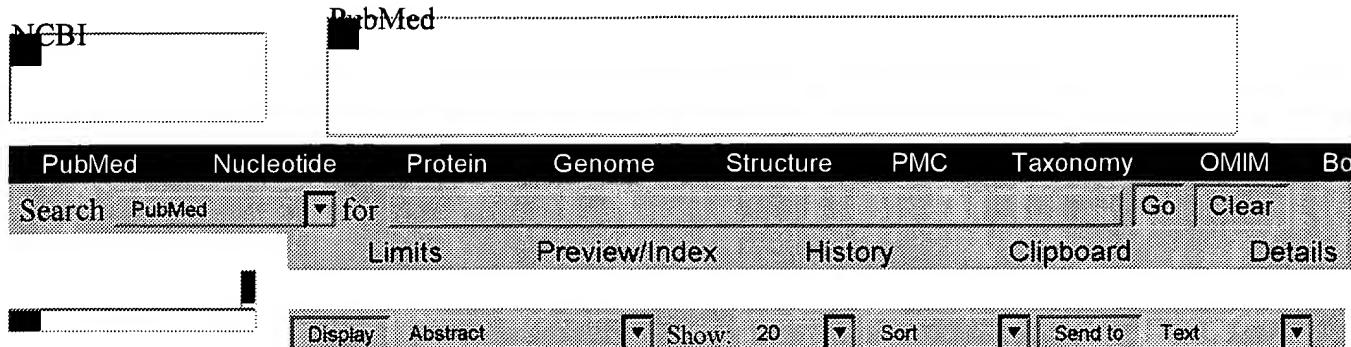
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Del Bufalo D, Di Castro V, Biroccio A, Varmi M, Salani D, Rosano L, Trisciuglio D, Spinella F, Bagnato A.

Experimental Chemotherapy Laboratories, Regina Elena Cancer Institute, Rome, Italy.

Related Resources

Endothelin-1 (ET-1) is a powerful mitogenic peptide produced by different tumors. In ovarian carcinoma cells, ET-1 acts as an autocrine growth factor, selectively through ET(A) receptor (ET(A)R), which is predominantly expressed in tumor cells. The aim of this study was to examine whether ET-1 plays a role in the sensitivity of three ovarian carcinoma cell lines (OVCA 433, HEY, and SK-OV-3) to apoptosis induced by two different stimuli. Our results demonstrated that the addition of ET-1 markedly inhibited serum withdrawal and paclitaxel-induced apoptosis in a concentration-dependent manner, as demonstrated by Annexin-V assay, sub-G(1) peak in DNA content histograms, internucleosomal DNA fragmentation, and terminal deoxynucleotidyl transferase-mediated dUTP biotin nick-end labeling method. Pretreatment of the cells with an ET(A)R antagonist, BQ 123, reversed the ET-1-induced protective effect. Paclitaxel-induced apoptosis resulted in the phosphorylation of Bcl-2 that was suppressed by the addition of ET-1. Further analysis of the signaling pathway demonstrated that ET-1 stimulated Akt activation. The phosphatidylinositol 3-kinase (PI3-K) inhibitor wortmannin blocked ET-1-induced Akt phosphorylation. Inhibition of ET-1-stimulated mitogen-activated protein kinase activity did not affect ET-1 protection from paclitaxel-mediated apoptosis. Moreover, BQ 123 blocked the Akt-mediated pathway activated by ET-1, sensitizing ovarian carcinoma cells to paclitaxel treatment. These results establish a novel role for ET-1 in determining protection of ovarian carcinoma cells against paclitaxel-induced apoptosis through Bcl-2-dependent and PI3-K-mediated Akt pathways and suggest that ET-1 and ET(A)R could represent important targets for anticancer therapy.



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Potential use of image analysis for the evaluation of cellular predicting factors for therapeutic response in breast cancers.

Bacus S, Chin D, Stewart J, Zelnick C, Mahvi D, Gilchrist K.

Advanced Cellular Diagnostics, Inc., Elmhurst, Illinois 60126, USA.

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OBJECTIVE: To summarize the current literature on the association between biologic prognostic factors and therapeutic implications. STUDY DESIGN: To illustrate how these biologic factors are determined and how they can affect treatment, three patients' biologic profiles and their implications for the patients' choice of therapeutic approaches were analyzed. Immunohistochemical techniques combined with image analysis was used to evaluate estrogen receptors, progesterone receptors, proliferation index and erbB-2. Visual assessment was used to evaluate P glycoprotein (MDR1), EGFR and p53. RESULTS: Data from the literature stress the importance of biologic profiles for defining tumor behavior and patient management. The examples of patients' biologic factors illustrated the possible importance of these factors for helping to design treatment. CONCLUSION: Today the data on the association of patient response to chemotherapy and molecular markers are only starting to accumulate. A larger database is needed for a more precise estimation of response probability in order to help physicians decide between treatment options.

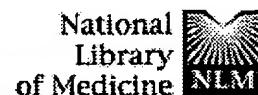
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The evaluation of estrogen receptor in primary breast carcinoma by computer-assisted image analysis.

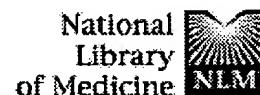
Bacus S, Flowers JL, Press MF, Bacus JW, McCarty KS Jr.

Cell Analysis Systems, Lombard, Chicago, Illinois.

A monoclonal antibody prepared against estrogen receptor has been shown to be specific and sensitive for the detection of estrogen receptor in human breast lesions by use of immunohistochemical methods. Two hundred selected cases of primary breast carcinoma were assayed for estrogen receptor content by biochemical and immunohistochemical procedures. Quantitative evaluation was by biochemical, immunohistochemical, and automated computer-assisted image analysis using the Cell Analysis System's CAS/100 machine (Lombard, IL). Quantitative estrogen receptor content was determined by dextran-coated charcoal analysis and sucrose density gradient analysis. Immunohistochemical evaluation incorporated both intensity and distribution of staining, yielding a subjective score, histologic score (HSCORE). An objective quantitation, also incorporating intensity and distribution of staining, was done by computer-assisted image analysis, quantitative immunocytochemical score (QIC SCORE). HSCORE analysis was done with and without methyl green counterstain with no loss of sensitivity. Comparison of QIC SCORE with the biochemical and immunohistochemical analysis of the tissues examined revealed excellent sensitivities and specificities. These data suggest that automated image analysis provides an effective qualitative and quantitative means of evaluating estrogen receptor content in human breast cancers.

PMID: 2458030 [PubMed - indexed for MEDLINE]

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1: *Pathol Annu*. 1993;28 Pt 1:179-204.

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Application of image analysis to the evaluation of cellular prognostic factors in breast carcinoma.

Bacus SS, Ruby SG.

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Cell Analysis Systems Reference Laboratory, Elmhurst, Illinois.

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Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen.

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Bacus SS, Kiguchi K, Chin D, King CR, Huberman E.

Cell Analysis Systems, Inc., Elmhurst, Illinois.

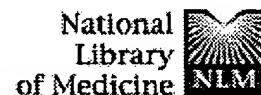
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The relationship between terminal cell differentiation and changes in the subcellular levels of the HER-2/neu antigen was investigated in cultured human breast cancer cells: AU-565 cells (which overexpress the HER-2/neu gene) and MCF-7 cells (which do not overexpress this gene). Differentiation was achieved by treating the cells with mycophenolic acid (MPA), phorbol 12-myristate 13-acetate (PMA), retinoic acid (RA), or the TA-1 monoclonal antibody to the extracellular domain of the HER-2/neu protein. Ten to twenty percent of the cells in untreated, sparsely growing AU-565 cultures exhibited morphological maturation characterized by large lacy nuclei surrounded by sizable flat cytoplasms. A fraction of these cells harbored milk factors such as casein and large lipid droplets. Treatment of the AU-565 cells for 4 d with 9 microM MPA, 1.6 nM PMA, 2.5 microM RA, or 1 microgram/mL TA-1 antibody resulted in cell growth inhibition and an increase in the percentage of cells (48-97%) that exhibit a mature phenotype. MCF-7 cells were also susceptible to differentiation by MPA and RA, but to a lesser degree than the AU-565 cells. Differentiation in the AU-565 and MCF-7 cells was associated with reduced immunostaining for the HER-2/neu protein at the cell surface membrane and with a transient increased diffuse immunostaining for this protein in the cytoplasm.

PMID: 1980588 [PubMed - indexed for MEDLINE]

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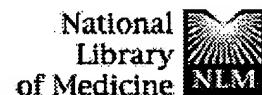
Tumor-inhibitory monoclonal antibodies to the HER-2/Neu receptor induce differentiation of human breast cancer cells.

Bacus SS, Stancovski I, Huberman E, Chin D, Hurwitz E, Mills GB, Ullrich A, Sela M, Yarden Y.

Cell Analysis Systems, Inc., Elmhurst, Illinois 60126.

Related Resources

The HER-2/neu protooncogene (also called erbB-2) encodes a tyrosine kinase receptor for a polypeptide growth-regulatory molecule. Amplification and overexpression of the gene have been frequently observed in human adenocarcinomas and correlated with poor prognosis. To explore the potential of antibody therapy directed at the HER-2/Neu receptor, we have raised a panel of murine monoclonal antibodies to the human protein, and tested their effect on the tumorigenic growth of HER-2/neu-transfected fibroblasts in athymic mice. We previously reported that the i.p. injected antibodies either inhibited or accelerated the tumorigenic growth of HER-2/neu transfectants in athymic mice. Here we report that these opposing effects were induced also by i.v. injected antibodies, they lasted over 7 weeks, and were probably mediated by distinct epitopes on the receptor molecule. To understand the cellular mechanisms underlying antibody-induced tumor inhibition, we tested the effect of the monoclonal antibodies on various cultured human breast cancer cells. Our analysis revealed that the tumor-inhibitory antibodies specifically induced phenotypic cellular differentiation that included growth arrest at late S or early G2 phase of the cell cycle, markedly altered cytoplasm and nuclear morphology, synthesis and secretion of milk components (casein and lipids), and translocation of the HER-2/Neu protein to cytoplasmic and perinuclear sites. The extent of cellular differentiation by various antibodies could be correlated with their tumor-inhibitory potential, whereas a tumor-stimulatory monoclonal antibody or control immunoglobulin were completely inactive with respect to cellular differentiation. Taken together, our *in vivo* and *in vitro* studies correlate the tumor inhibitory potential of monoclonal antibodies to HER-2/Neu with their capacity to induce cellular differentiation *in vitro*. This observation may hold promise for immunotherapy of cancers that express the HER-2/neu oncogene.



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Advanced Cellular Diagnostics, Inc., Elmhurst, Illinois 60126, USA.

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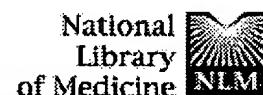
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J Biol Chem. 2003 Sep 2 [Epub ahead of print]
PMID: 12952968 [PubMed - as supplied by publisher]

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The PI3-kinase-Akt pathway promotes mesangial cell survival and inhibits apoptosis in vitro via NF-kappa B and Bad.
J Am Soc Nephrol. 2003 Jun;14(6):1427-34.
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Cancer Immun. 2001 Jul 13;1:8.
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Bcl-xL mediates a survival mechanism independent of the phosphoinositide 3-kinase/Akt pathway in prostate cancer cells.
J Biol Chem. 2003 Jul 11;278(28):25872-8. Epub 2003 May 08.
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Androgen-independent growth of LNCaP prostate cancer cells is mediated by gain-of-function mutant p53.
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J Biol Chem. 2003 Jun 13;278(24):21869-77. Epub 2003 Apr 02.
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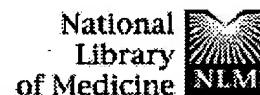
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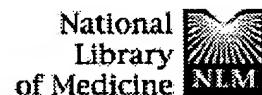
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